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A DNA nanocapsule with aptamer-controlled open-closure function for targeted delivery

Thomas Bentin

Department of Cellular and Molecular Medicine; University of Copenhagen; Copenhagen, Denmark

A DNA capsule fitted with aptamer controlled target sensing has been "woven" using a 7308-base single-stranded DNA "thread" and 196 staple oligonucleotides. The capsule enables logic-gated molecular cargo delivery to targeted cell surfaces.

Using DNA origami,[1] the group of Georg Church has engineered a capsule that can be loaded with molecular cargo and unloaded upon binding to a desired target cell.[2] The capsule was designed using computer software, to have the shape of a hexagonal barrel with open ends, and dimensions of 35 × 35 × 45 nm. Each ~2.4 megadalton half-barrel is connected covalently via DNA hinges at the rear. At the front, locking and unlocking is accomplished by the making and breaking of short DNA duplexes at the left and right anterior of the capsule. Each duplex lock contains an aptamer attached to one half-barrel domain, which in the locked conformation is hybridized to a partially complementary strand connected with the other half-barrel domain. When presented with its molecular target (coined antigen-key), the aptamer-complement duplexes dissociate forming aptamer-antigen-key complexes. Simultaneous dissociation of both aptamer-complement duplexes unlocks the capsule, which open due to the entropic penalty of capsule closure combined with phosphate backbone repulsion between half-barrel domains thus exposing its previously hidden inner surfaces. Experiments using aptamer-complement duplex lengths of 16–44 bp revealed that the optimal lock length tested (23 bp) was a compromise between reducing spontaneous opening while maintaining sensitivity and speed of activation in the absence and presence of antigen-key, respectively. Using the above described design, only 48% of the capsules could be closed. Capsule closure was further enhanced to 97.5% using additional guide staple strands that were subsequently removed.

To enable cargo loading, short oligonucleotides were conjugated to the cargo, and capsule staples were correspondingly modified with complementary sequences at multiple internal attachment sites. Theoretical considerations indicated substantial diffusion of appropriately sized molecules through the open ends of the capsule barrel structure. Thus, capsules were loaded in their closed barrel conformation (simplifying handling) with efficiencies averaging three Fab' antibody fragments (range 0–5) or four 5 nm gold nanoparticles (range 0–7) per capsule.

To assay capsule function, different cancer cells were exposed to capsules loaded with fluorescently labeled antibody fragments against human leukocyte antigen (HLA)-A/B/C. These cells all expressed (HLA)-A/B/C and different antigen-key combinations. Consequently, capsule unlocking required the correct aptamer-key combination and once unlocked, target cells were labeled by the cargo. Using this approach, six aptamer-lock capsule combinations were tested against six different cell types. Only cells expressing correct keys promoted capsule opening. Impressively, capsules equipped with two different aptamer-locks correctly required the corresponding two surface antigen-keys for activation. In other words, the capsules behaved as logical

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Correspondence to: Thomas Bentin; Email: bentin@sund.ku.dk

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AND gates where an output requires two separate simultaneous inputs.

To investigate target specificity further, capsules were challenged with mixed cell populations. In mixed Ramos/NKL$^{-/+}$ antigen-key expressing cell populations (ratio 2:1), the target NKL cells were selectively labeled. Similarly, in whole blood/NKL mixtures (ratio 4:1) the NKL cells were almost exclusively labeled (0.6% non-target cell labeling). In Jurkat/Ramos cell compositions, the target Jurkat cells were correctly singled out in the range $10^6/10^6$ down to $1/10^6$ cells.

To explore whether capsules could be used to mediate meaningful biological signaling, bio-active cargo was used. Capsules loaded with growth arrest promoting antibody fragments induced growth arrest in leukemic cells. Capsules loaded with T-cell activating antibody fragments promoted T-cell activation, and enhanced T-cell activation was achieved by additional capsule loading with peptide (binding to one of the already loaded antibody fragments) known to stimulate activation. Consequently, these capsules may be used to impact cell physiology.

Others have built comparable structures[3] but the aptamer-lock/capsule combination used by Douglas and coworkers[2] appears to effectively harness the potential of both the nano and aptamer world in promoting targeted delivery. Their capsules respond to predefined cell surface cues and specifically single out the desired target cells, and as pointed out by the authors, with multivalent payload delivery. Together, the presently described achievements (see ref. 2) increase prospects for a "magic bullet" type delivery system that improves specificity and reduces toxicity of certain drugs.

To this end, several questions remain unanswered. The toxicity of the vehicle itself remains unknown. The lifetime of the intact closed capsule state in humans is unknown; important in preventing premature cargo access. Capsule stability might be compromised during hepatic passage. The aptamer-complement locks in particular would appear vulnerable to exonuclease digestion. Simple lock modification with synthetic DNA analogs could help ameliorate such potential problems.

Functionally, the DNA capsules resemble well-described lipid-based nanoparticles, which are already used in the clinic for targeted delivery in cancer therapy.[4] There are important differences though. Whereas the present capsules deliver their cargo to the cell surface, lipid-based nanoparticles pass through the endocytotic pathway. Thus DNA capsules may be useful when using surface active or cell permeable compounds whereas liposomal vehicles are preferred with compounds that have intracellular targets and are cell impermeable (e.g., siRNA). Additional basic research is required to explore the potential of aptamer controlled DNA capsules but the present advancements provide a great leap toward a practical application.

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